Technische Universiteit
Eindhoven
University of Technology

**iGEM TU/e 2016**
Biomedical Engineering

Eindhoven University of Technology
Room: Ceres 0.04
Den Dolech 2, 5612 AZ Eindhoven
The Netherlands
Tel. no. +31 50 247 55 59
2016.igem.org/Team:TU_Eindhoven

# Preparation of general necessities

Where innovation starts

Technische Universiteit
Eindhoven
University of Technology

# Table of contents

Preparation of general necessities

# 1 Preparation of Culture Media

## 1.1 Luria-Bertani (LB) Medium

**Estimated bench time:** 15 minutes
**Estimated total time:** 3 hours
**Purpose:** This medium is used for small cultures of competent cells containing desired DNA.

### 1.1.1 Materials

- Autoclave
- Autoclave tape
- Balance
- Bottle (glassware)
- $dH_2O$
- NaCl (Sodium chloride)
- Peptone
- Yeast extract

### 1.1.2 Protocol

- For 1 l LB medium, the correct amounts are:
  - 10 g NaCl
  - 10 g peptone
  - 5 g yeast extract
- Collect them in in a bottle and add 1 l of $dH_2O$.
- Autoclave the LB medium at 121 °C for 20 minutes (sterilisation).

## 1.2 2YT

**Estimated bench time:** 15 minutes
**Estimated total time:** 3 hours
**Purpose:** This medium is used for the protein expression of competent cells with more than one plasmid.

### 1.2.1 Materials

- Autoclave
- Autoclave tape
- Balance
- Bottle (glassware)
- $dH_2O$
- NaCl (Sodium chloride)
- Peptone
- Yeast extract

### 1.2.2 Protocol

- For 1 l LB medium, the correct amounts are:
  - 10 g yeast extract
  - 16 g peptone
  - 5 g NaCl
- Collect them in in a bottle and add 1 l of $dH_2O$.
- Autoclave the 2YT at 121 °C for 20 minutes (sterilisation).

# 2 Agar Plates

## 2.1 Preparation of LB-agar

**Estimated bench time:** 15 minutes
**Estimated total time:** 3 hours
**Purpose:** Making LB-agar for the pouring of LB-agar plates.

### 2.1.1 Materials

- Autoclave
- Autoclave tape
- Bacto-agar
- Balance
- Bottle (glassware)
- $H_2O$
- NaCl (Sodium chloride)
- Peptone
- Yeast extract

### 2.1.2 Protocol

With 200 ml LB-agar you can make 8 plates (1 l for ~40 plates).

- For 200 ml LB-agar, the correct amounts are:
  - 1 g yeast extract
  - 2 g NaCl
  - 2 g peptone
  - 3 g bacto-agar
- Collect them in in a bottle and add 200 ml of $H_2O$.
- Autoclave the LB-agar at 121 °C for 20 minutes (sterilisation).

## 2.2 Pouring the plates

**Estimated bench time:** 20 minutes
**Estimated total time:** 80 minutes
**Purpose:** Making LB-agar plates where bacteria with an additional plasmid can grow.

It is essential to work sterile, thus disinfect your hands and work near a Bunsen Burner.

### 2.2.1 Materials

- Antibiotic stock
- Autoclaved LB-agar
- Bunsen burner
- Petri-dishes
- Pipettes and tips

### 2.2.2 Protocol

- After autoclaving the LB-agar (at 121 °C for 20 minutes), let the agar cool down to ~50 °C (autoclave can be opened at 90 °C). Make sure the agar does not start solidifying.
- Add antibiotic stock (200 µl for 200 ml) to the liquid LB-agar and slowly mix.
- Pour the LB-agar in the petri-dishes until the bottom is well covered. Work near the Bunsen burner flame.
- Close the lid after filling the plate. Let the agar solidify for ~1 hour on the bench.
- Transfer the plates to a bag, in which they should be placed upside down.
- Store the plates in the fridge (4 °C).

# 3 Antibiotic stocks

## 3.1 Kanamycin stock (50 mg/ml)

**Estimated bench time:** 15 minutes
**Estimated total time:** 15 minutes
**Purpose:** Making antibiotic stock, necessary for LB-agar plates and the LB medium for small culturing.

Protocol for 10 ml kanamycin stock (50mg/mL)
Final concentration in cultures and LB-agar: 30 µg/mL
Aliquot in Eppendorf tubes, 1 mL per tube.

### 3.1.1 Materials

- 0.22 µm filter
- Kanamycin sulfate powder
- Balance
- Eppendorf tubes
- Falcon tube
- MiliQ
- Syringe
- Vortex

### 3.1.2 Protocol

- Dissolve 500 mg kanamycin sulfate powder in 10 mL MiliQ.
- Mix/vortex so that all the kanamycin goes into solution.
- Filter into a falcon tube using a syringe and a 0.22 µm filter for sterilization.
- Aliquot into smaller Eppendorf tubes.
- Store at -20 $^{0}$C under dark conditions.

## 3.2 Ampicillin stock (35 mg/ml)

**Estimated bench time:** 15 minutes
**Estimated total time:** 15 minutes
**Purpose:** Making antibiotic stock, necessary for LB-agar plates and LB medium for small culturing.

### 3.2.1 Materials

- 0.22 µm filter
- Balance
- Ampicillin
- Eppendorf tubes
- Ethanol (100%)
- Falcon tube
- Syringe
- Vortex

### 3.2.2 Protocol

# 4 General necessities

## 4.1 50% Glycerol

**Estimated bench time:** 10 minutes
**Estimated total time:** 3 hours
**Purpose:** Preparing glycerol for a glycerol stock, which can be made for long term storage of bacteria.

### 4.1.1 Materials

- Autoclave
- Autoclave tape
- Balance
- Bottle (glassware)
- Glycerol (100%)
- MilliQ

### 4.1.2 Protocol

- Add 10 ml of glycerol (100%) to a bottle.
- Add 10 ml of MilliQ to the glycerol.
- Autoclave the glycerol stock at 121 °C for 20 minutes (sterilisation).

## 4.2 Sterile $H_2O$

**Estimated bench time:** 5-10 minutes
**Estimated total time:** 3 hours
**Purpose:** Making sterile $H_2O$ (nuclease free water), necessary for making dilutions.

Aliquot needs to be done sterile, thus disinfect your hands and work near a Bunsen Burner.

### 4.2.1 Materials

- Autoclave
- Autoclave tape
- Bottle (glassware)
- Bunsen Burner
- Eppendorf tubes (sterile)
- MilliQ
- Pipette and tips

### 4.2.2 Protocol

- Fill a bottle with 100 ml MilliQ.
- Autoclave the MiliQ at 121 °C for 20 minutes (sterilisation).
- Optional: aliquot a part into sterile Eppendorf tubes for easy usage.

## 4.3 Sterile Eppendorf, PCR and sequencing tubes

**Estimated bench time:** 10 minutes
**Estimated total time:** 3 hours
**Purpose:** Making the Eppendorf tubes, PCR tubes and sequencing tubes sterile so that they can be used during experiments.

### 4.3.1 Materials

- Autoclave
- Autoclave tape
- Beaker
- Eppendorf tubes
- PCR tubes
- Sequencing tubes
- Tin foil

### 4.3.2 Protocol

- Fill a beaker with the tubes, for each type, use another beaker.
- Cover the upside with tin foil.
- Autoclave the beakers with the tubes at 121 °C for 20 minutes (sterilisation). Use a dry sterilization program or let the tubes dry in an incubator set at 50 °C.

## 4.4 Glycerol stock

**Estimated bench time:** 5 minutes per sample
**Estimated total time:** 10 minutes per sample
**Purpose:** Preparing the bacteria for long term storage in the -80 °C freezer.

It is essential to work sterile, thus disinfect your hands and work near a Bunsen Burner.
For working with liquid nitrogen it is important to wear Cryo gloves.

### 4.4.1 Materials

- Autoclaved glycerol (50%)
- Bacterial cultures
- Bunsen Burner
- CryoTubes
- Pipettes and tips

### 4.4.2 Protocol

- Fill CryoTubes with 300 µl 50% glycerol and 700 µl of the bacterial culture. Mix well.
- Snap freeze the samples in liquid nitrogen and transfer them to the -80 °C freezer.